*Short Communication*

# Long-term Storage and Recovery of Buccal Cell DNA from Treated Cards

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## Abstract

**Economical methods for collecting and storing high-quality DNA are needed for large population-based molecular epidemiology studies. Buccal cell DNA collected via saliva and stored on treated filter paper cards could be an attractive method, but modest DNA yields and the potential for reduced recovery of DNA over time were unresolved impediments. Consequently, buccal cell DNA collection via oral mouthwash rinsing became the method of choice in epidemiologic studies. However, the amount of genomic DNA (gDNA) required for genotyping continues to decrease, and reliable whole genome amplification (WGA) methods further reduced the mass of gDNA needed for WGA to 10 ng, diminishing the obstacle of low DNA yields from cards. However, concerns about yield and DNA quality over time remained. We located and analyzed 42 buccal cell saliva samples collected and stored on treated cards for 7 years at room temperature, −20°C, and −80°C. We recovered DNA from the treated cards, estimated the concentration by a human-specific quantitative real-time PCR assay, and evaluated the quality by PCR amplification of 268-, 536-, and 989-bp fragments of the *β-globin* gene and by AmpF*l*STR Identifiler assay analysis. Most DNA yields per 3-mm punch were <10 ng, and most PCR amplicons failed to amplify, where size of the amplicon was negatively associated with successful amplification. Using these methods, treated cards did not consistently provide sufficient quantities of buccal cell gDNA after 7 years of storage for genotyping or WGA.** (Cancer Epidemiol Biomarkers Prev 2006;15(2):385–8)

## Introduction

The growing use of whole genome amplification (WGA) methods to faithfully increase genomic DNA (gDNA) mass (1-3) and the high costs of collecting and processing blood or mouthwash samples inspired renewed consideration of treated filter paper cards as a method to collect DNA from buccal swabs. Foam-tip swabs, rubbed along the inner cheek and gum, become saturated with saliva and exfoliated cells and can be pressed onto filter paper cards that have been pretreated to retard bacterial growth, inhibit nuclease activity, and release DNA during processing (Isocode, Schleicher and Schuell, Keene, NH). The swabs and Isocode cards may be easier to use in pediatric (1) and elderly (4) populations to collect specimens and can be mailed in an envelope with a desiccant at a nominal cost. Although lower DNA yields are obtained from buccal brushes or swabs than mouthwash samples (reviewed in ref. 5), the costs per person using swabs can be less than half (US$8.50) than those for mouthwash (US$18; ref. 6). However, little information was available to evaluate DNA quantity and quality from treated cards after storage for >5 years. One study reported success with PCR-based assays over 3 years from cytobrush samples, but these had been processed before storage (7), and two other studies successfully analyzed short tandem repeat (STR) markers after storing saliva on treated cards for 5 months (8) or filter paper for 2 years (9). Ideally, using treated cards, saliva collected prospectively could be economically and indefinitely stored until sufficient numbers of diseased individuals are accrued to conduct nested case-control studies. At that time, gDNA could be eluted and genotyped or undergo whole genome amplification from the card (at an approximate cost of US$4-6 each) and the resultant whole genome amplified DNA (wgaDNA) in ample quantities could be used for genotyping. We sought to evaluate the amount and quality of buccal cell DNA obtained 7 years after collection from treated cards that were stored with a desiccant at room temperature, −20°C and −80°C. We used a subset of samples remaining from the report of Harty et al. (10) that investigated buccal cell DNA quantity, quality, and stability after 1 week and 9 months of storage at three different temperatures.

## Materials and Methods

**Subjects.** At the time of the original study, 52 subjects provided written informed consent and the study protocol was approved by the human subjects review boards of the National Cancer Institute and Westat, Inc. (10). Buccal cells were

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collected between March 20, 1997 and April 3, 1997. For the present study, all sample materials were anonymized, and the participant identities were unknown to the investigators. Portions from 42 of the 52 original cards were located and used for this study.

**Sample Collection and Storage.** Original sample collection and handling methods have been reported previously in Harty et al. (10). The original protocol evaluated freshly collected cards as well as cards stored at three temperatures (room temperature, −20°C, and −70°C) for 9 months. The cards remained thereafter at similar temperatures over the ensuing 7 years. The 42 sample cards were retrieved, and 3-mm circles were punched in each card within the embossed circle (where the highest concentration of DNA had been reported; ref. 10) at the 12, 3, and 9 o'clock positions. The paper puncher was flame sterilized between samples. The 12 and 3 o'clock punches were sent to the American Type Culture Collection (Manassas, VA) facility where the original study DNA elutions had been done. The 9 o'clock punch was sent to the National Cancer Institute's Core Genotyping Facility for processing, multiplex genotyping, and genotyping after WGA. All the unused portions of the cards were returned to their respective storage temperatures.

**Sample Processing.** The two punches obtained from the 12 and 3 o'clock card positions were subjected to three separate elutions to assess the possibility of DNA fixation to the card and the effects of each elution on *β-globin* gene fragment size. These samples were processed as follows (DNA recovery method 1): both discs of treated paper were washed in molecular biology grade water that was next removed. Then, 60 μL of molecular biology grade water were added to the discs, and the samples were denatured at 95°C for 15 minutes, pulse vortexed, denatured again for 15 minutes at 95°C, and pulse vortexed again. The fluid containing the DNA was transferred to a new microcentrifuge tube (elution 1). The elution process was repeated twice to create elution 2 and elution 3. The punch obtained from the 9 o'clock position was processed as follows (DNA recovery method 2): the punch and 30 μL of water were added to each well in the plate and denatured at 95°C for 15 minutes, vortexed, and denatured again at 95°C for 15 minutes. The plates were centrifuged, and three 2.0 μL of sample were removed to establish three replicate samples.

**DNA Quantification and Quality Assessment.** A quantitative real-time PCR assay (11) was done on all DNA samples to estimate the total human-specific PCR amplifiable DNA yield from each elution. Quantitative real-time PCR assay performance was compared and normalized across laboratories using concentration estimates from a set of $n = 28$ control DNA samples done in both laboratories (Spearman $\rho = 0.974$, $P < 0.0001$). All comparisons were adjusted per one punch in 30 μL.

Three regions of the *β-globin* gene of 268, 536, and 989 bp were amplified as described (10) for the three elutions and each storage temperature (room temperature, −20°C, and −80°C). Multiplex genotyping of 15 STR regions and the amelogenin locus (gender determination) was done using the AmpF*l*STR Identifiler assay (Applied Biosystems, Inc., Foster City, CA) as described (12), except using either 1.25 ng or 2.0 μL of sample when the DNA sample concentration was <0.625 ng/μL.

**Statistical Analysis.** We assessed several indicators of DNA quantity and quality after storage for 7 years at three different temperatures. We calculated the proportion of samples with no recoverable DNA and the proportion of samples with <10 ng of DNA, generally considered the lower limit for successful and reproducible WGA without significant allelic amplification bias (2, 13). Significant differences in proportions were assessed by contingency table analysis (Pearson's $\chi^2$ test and Fisher's exact test). Descriptive statistics (mean, median, and range) were used to assess nonzero DNA yields (in ng) by elution and storage temperature and by replicate and storage temperature per 3-mm punch. All statistical tests were two sided and were done using PC-SAS version 8.02 (SAS Institute, Inc., Cary, NC).

## Results

DNA yields per punch and the proportion of samples with no detectable DNA yield or yields <10 ng calculated from each elution (DNA recovery method 1), 2-μL replicate aliquot (DNA recovery method 2), and storage temperature are shown in Table 1. For DNA recovery method 1, elution 1 tended to provide the largest amount of DNA (data not shown) among nonzero samples, and samples stored at room temperature summed over three elutions contained the most DNA compared with those stored at −20°C or −80°C. Room temperature samples also had the lowest proportion with DNA yields <10 ng (38%) compared with −20°C (62%) or −80°C (48%; $P = 0.09$). For DNA recovery method 2, the averaged mean DNA yield of three replicates was highest for samples stored at −20°C. DNA recovery method 1 had a lower proportion of samples with no detectable DNA and a lower proportion of DNA samples with <10 ng DNA than did DNA recovery method 2 but had lower mean and median yields than did DNA recovery method 2.

Successful PCR amplification of *β-globin* gene fragments was significantly associated with room temperature storage, Elution 1, and the shorter 268-bp fragment, where the three amplicons exhibited mean success rates across the three elutions of 55.8%, 18.8%, and 4.8%, respectively (Table 2). As the number of elutions and the *β-globin* gene fragment size increased, the proportion of successfully amplified samples

**Table 1. Human DNA yields from 3-mm punches of buccal cells stored on treated cards for ~7 years at three temperatures (room, −20°C, and −80°C) using real-time PCR by two DNA recovery methods**

| | DNA recovery method 1* ($n = 42$) | DNA recovery method 2† ($n = 42$) |
|---|---|---|
| Room temperature | | |
| Mean in nonzero samples (ng) | 28.6 | 46.6 |
| Median in nonzero samples (ng) | 19.7 | 14.8 |
| Range in nonzero samples (ng) | 0.4-121.9 | 0.1-489.9 |
| No detectable DNA, *n* (%) | 1 (2.4%) | 13 (31.0%) |
| <10 ng of DNA, *n* (%) | 16 (38.1%) | 26 (61.9%) |
| −20°C | | |
| Mean in nonzero samples (ng) | 19.9 | 81.3 |
| Median in nonzero samples (ng) | 8.4 | 9.6 |
| Range in nonzero samples (ng) | 0.4-177.4 | 0.0-877.9 |
| No detectable DNA, *n* (%) | 3 (7.1%) | 18 (42.9%) |
| <10 ng of DNA, *n* (%) | 26 (61.9%) | 30 (71.4%) |
| −80°C | | |
| Mean in nonzero samples (ng) | 20.1 | 65.6 |
| Median in nonzero samples (ng) | 10.9 | 17.5 |
| Range in nonzero samples (ng) | 0.4-126.2 | 0.0-578.3 |
| No detectable DNA, *n* (%) | 5 (11.9%) | 22 (52.4%) |
| <10 ng of DNA, *n* (%) | 20 (47.6%) | 31 (73.8%) |
| *P* for % with no DNA‡ | 0.29§ | 0.14 |
| *P* for % with <10 ng DNA‡ | 0.09 | 0.46 |

NOTE: Human DNA yield per punch in ng was adjusted to the same volume (30 μL) and also adjusted for DNA quantification differences by laboratory. The American Type Culture Collection laboratory's DNA quantitation (DNA recovery method 1) was 1.4 times that of the National Cancer Institute's Core Genotyping Facility (DNA recovery method 2); thus, method 1 yields were divided by 1.4.

*Two punches, 60-μL elution, three separate elutions. Amounts were summed over the three elutions.

†One punch, 30-μL elution, three replicate 2-μL aliquots. Amounts were averaged over the three replicates.

‡*P* for $\chi^2$ over all three storage temperatures.

§Fisher's exact test.

**Table 2. Number of samples with successful PCR amplification of *β-globin* gene fragments from buccal cell DNA stored for ~7 years on treated cards by elution number and storage temperature (DNA recovery method 1)**

| | Storage temperature | Elution 1 ($n$ = 42) | % Success | Elution 2 ($n$ = 42) | % Success | Elution 3 ($n$ = 42) | % Success | $P$ |
|---|---|---|---|---|---|---|---|---|
| *β-globin* gene | | | | | | | | |
| 268 bp (98.1%)* | Room | 39 | 92.9 | 35 | 83.3 | 21 | 50.0 | <0.0001 |
| | −20°C | 28 | 66.7 | 16 | 38.1 | 8 | 19.1 | <0.0001 |
| | −80°C | 30 | 71.4 | 25 | 59.5 | 9 | 21.4 | <0.0001 |
| $P$ | | | 0.01 | | 0.0001 | | 0.003 | |
| 536 bp (98.1%)* | Room | 21 | 50.0 | 12 | 28.6 | 3 | 7.1 | <0.0001 |
| | −20°C | 6 | 14.3 | 2 | 04.8 | 8 | 19.1 | 0.13 |
| | −80°C | 14 | 33.3 | 5 | 11.9 | 0 | 0.0 | <0.0001 |
| $P$ | | | 0.002 | | 0.71 † | | 0.007 † | |
| 989 bp (92.3%)* | Room | 6 | 14.3 | 4 | 9.5 | 1 | 2.4 | 0.18 † |
| | −20°C | 1 | 2.4 | 2 | 4.8 | 0 | 0.0 | 0.77 † |
| | −80°C | 1 | 2.4 | 3 | 7.1 | 0 | 0.0 | 0.33 † |
| $P$ | | | 0.049 † | | 0.91 † | | 1.00 † | |

*Comparisons are shown in parentheses for successful amplification with 52 fresh samples from Harty et al (10) using Taq polymerase or AmpliTaq Gold for 268-, 536-, and 989-bp fragments.
† Fisher's exact test.

decreased. The proportions of samples failing STR genotyping by the type of DNA recovery method were 76.0%, 92.4%, and 94.2% for DNA recovery method 1 and 85.6%, 93.0%, and 89.1% for DNA recovery method 2 for room temperature, −20°C, and −80°C storage conditions, respectively. DNA from cards stored at room temperature exhibited significantly lower STR failure rates than those stored frozen ($P < 0.0001$ for both methods).

## Discussion

Buccal cells collected on treated cards could be an economical collection and storage methodology for field-based epidemiologic and other large studies (1, 4, 6, 9, 10). Researchers might be willing to tolerate a low level of sample degradation or loss years later, as these losses may not differentially affect cases and controls for diseases of interest. We have recovered and analyzed buccal cell DNA stored on treated cards for ~7 years at three temperatures using quantitative real-time PCR and PCR-based assays for nonpolymorphic and polymorphic human loci. Although storage temperature and DNA recovery method did affect the DNA yield from the treated cards, we found the cards did not provide suitable quantities or quality of DNA after 7 years. Except for DNA recovery method 1 among samples stored at room temperature, the proportion of samples with no detectable DNA yield exceeded 7% using both DNA recovery methods, and most eluates yielded <10 ng DNA. STR genotyping failed in ≥76% of the samples and detection of *β-globin* gene fragments ≥536 bp was <50%. For samples stored at room temperature, the 268-bp fragment was successfully amplified in 93% of samples. Successful amplification for these stored samples was less than that for fresh samples (previously, over 92% of the fresh samples successfully amplified for all the fragment sizes; ref. 10), but the DNA degradation observed indirectly in the present study, after 7 years of storage, was greater than expected.

We attempted to determine the reasons for reduced DNA yield, and we found evidence for both DNA fixation to the card and degradation over time. Multiple elutions (up to three, which included heating to 95°C each time) produced additional but more degraded DNA. However, even when the fragment size analysis was restricted to elution 1, there was clear evidence that the larger fragment sizes also failed to amplify, suggesting the DNA had degraded over 7 years. It may be that the DNA amount varied across the card within the embossed circles or that DNA yields and successful amplification would improve if the entire circle were processed. We did not attempt processing the entire circle as per agreements with the original study investigators to leave some portion unused.

It is possible that buccal cell DNA could be collected on treated cards by mail, processed within a few weeks, aliquoted, and the DNA stored in appropriate long-term freezer vials. We did not assess the feasibility of this option nor did we assess DNA yield had the entire card undergone processing. Nevertheless, impractically low DNA yields could be overcome by WGA methods (1-3, 13), as necessary, selecting WGA methods optimized for degraded samples (14, 15). Researchers should also be aware that post offices in selected geographic areas (those government offices served by 202 to 205 zip codes) now employ electron-beam irradiation of the U.S. mail as a sterilant, rendering buccal cell DNA slightly compromised for genotyping (12) and unsuitable for WGA that requires high-molecular weight DNA as template (16).

In summary, we found that buccal cell DNA quantity and quality were seriously compromised after 7 years of storage on treated cards, and this method would not be suitable for large prospective epidemiologic studies where the disease of interest, such as cancer, might be relatively rare and could take years to occur. Quantity and quality were generally worse if the samples had been stored at −20°C or −80°C compared with room temperature. Despite these limitations, the prospect of WGA coupled with small amounts of DNA collected by mail from simple self-administered buccal mucosa swabbing is still feasible if the long-term stability issue can be overcome or if processing of the DNA from the collection medium occurs promptly after collection.

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